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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 7/16	A1	(11) International Publication Number: WO 90/10434
		(43) International Publication Date: 20 September 1990 (20.09.90)

(21) International Application Number: PCT/SE90/00165

(22) International Filing Date: 15 March 1990 (15.03.90)

(30) Priority data:
8900915-3 15 March 1989 (15.03.89) SE

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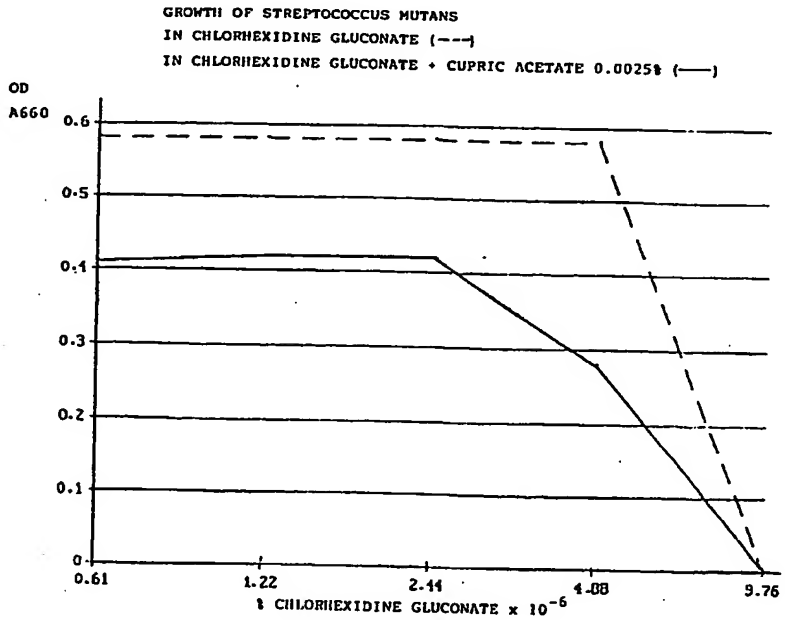
(81) Designated States: AT (European patent), AU, BE (European patent), BG, BR, CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), HU, IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, RO, SE (European patent), SU, US.

Published
With international search report.

(54) Title: DENTAL PREPARATION



(57) Abstract

A dental preparation containing a water soluble, physiologically acceptable salt of an antimicrobal bisbiguanide, preferably chlorhexidine, and a water soluble physiologically acceptable cupric salt presents a synergestic plaque inhibiting effect in combination with a reduced discoloration and calculus formation. The dental preparation can be in the form of a mouth rinse, toothpaste or gel, or as a tablet r a chewing gum.

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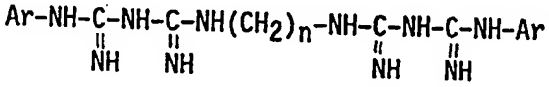
Dental preparation

The present invention refers to a dental preparation containing a water soluble, physiologically acceptable salt of an antimicrobal bisbiguanide. Said dental preparation can be in the form of a mouth rinse, tooth paste, gel, tablet or chewing gum, which prevents plaque formation on the teeth. By this the development of caries and/or periodontitis, that is loosening of the teeth, can be prevented.

It is known that several bisbiguanides show an antimicrobal effect. Said bisbiguanides have the general formula



wherein Ar is an optionally substituted aryl group and n is an integer from 4 to 8, especially 6. Several of these compounds have been tested and the compound having the broadest field of use is chlorhexidine.

Chlorhexidine is difficult to dissolve in water and has to be transformed into a salt to attain a soluble form. The salts which have been found to be appropriate to use as an antimicrobal preparation are particularly the acetate and the gluconate. The gluconate salt of chlorhexidine in particular has been used extensively and is inter alia sold by ICI, Machlefield, England under the trademark "HIBITANE".

It is well known that chlorhexidine and in particular "HIBITANE" can prevent the formation of bacterial deposits (plaque) on the teeth of individuals rinsing the mouth twice daily with for instance a 0.1-0.2 % by weight solution of "HIBITANE". It is also well established that the clinical effect of lower concentrations of chlorhexidine is negligible.

The bacterial deposits on the teeth is a prerequisite for both caries and periodontitis. Caries depends on the formation of lactic acid in connection with the consumption of sugar while periodontitis arises in connection with the accumulation of anaerobic, toxin forming bacteria on the teeth. Rinsing twice daily with chlorhexidine consequently prevents both caries and periodontitis, which has been shown in tests on animals as well as in clinical tests.

Chlorhexidine also arrests the development of dental diseases already started. In addition to be used in mouth rinses chlorhexidine can also be used in the form of tooth paste or gel for tooth brushing, giving the same

effect as previously stated.

It could be expected that these chlorhexidine salts and similar bis-biguanides, owing to said favourable characteristics, should have been extensively used within odontology and dental care. A serious restriction of these preparations is however the important discoloration of the teeth after using chlorhexidine or other similar bisbiguanide salts after a certain time. The discoloration is specially formed between the teeth, is dark brown to black in color and hard to remove as it is calcified. The discoloration probably consists of calcified pellicle protein which has been discolored by metal sulfides, especially iron, from the food. The discoloration probably is formed by denaturing of the pellicle protein, which covers the surfaces of the teeth with a thin layer, by the bisbiguanide. Exposed sulphur groups then bind metals which are thus discolored, and all of the pellicle mass is then calcified on a long due.

It has since long been desirable to use the favourable plaque preventing properties of chlorhexidine and other bisbiguanide salts at the same time as said discoloration is avoided.

DE-A1-25 34 887 refers to a dental preparation with inhibiting effect on the formation of plaque and caries, comprising in addition to for instance chlorhexidine a chelate forming compound. The purpose of the chelate forming compound is to prevent the discoloration of the teeth which is brought about by chlorhexidine. Preferred chelate forming compounds are ethylene diamino-acetic acid, kojic acid, malthol and calcium dihydrogen ethylenedi-aminotetraacetate.

Another problem with chlorhexidine in dental preparations is the increased calculus formation which can be observed after use for some time.

Chlorhexidine also, when used as a mouth rinse or tooth paste, can bring about a temporary loss of taste sensation, which seems to be dependent on the concentration.

Copper(II)compounds, that is the cupric ion, are previously known to inhibit plaque formation. In J. of Clinical Periodontology 1984, 11, p. 176-180, Waerhaug M. et al have compared the effect of chlorhexidine and the effect of $CuSO_4$ on plaque formation and development of gingivitis. Although $CuSO_4$ showed a significant inhibiting effect, said effect was not as marked as that of chlorhexidine. It was noted that the test subjects complained about the taste of the test substances. Cupric sulphate seems to be more acceptable than chlorhexidine in this respect and did not cause any lasting interference with the taste sensation.

It has now been discovered that the favourable plaque preventing properties of bisbiguanide salts, such as chlorhexidine acetate and gluconate, can be utilized in full and the formation of discolorations and calculus on the teeth reduced and the loss of taste sensation prevented by means of a dental preparation, such as a tooth paste, mouth rinse, tablet or chewing gum, which in addition to a water soluble, physiologically acceptable salt of an antimicrobal bisbiguanide, contains a water soluble, physiologically acceptable cupric salt.

It has also surprisingly been found that a combination of a bisbiguanide and a cupric salt, such as cupric acetate or cupric gluconate, has a synergistic plaque inhibiting effect, at the same time as the formation of discoloration is mild and of such a character, that it can easily be brushed away by the patient by means of tooth brushing, which is not possible with discolorations which have been formed in connection with the use of chlorhexidine solely.

The dental preparation of the invention comprises the salt of the bisbiguanide and the cupric salt in a proportion giving a synergistic plaque preventing effect.

It is believed that the calculus preventing effect of the preparation of the invention could be ascribed to a mechanism in which Cu^{2+} is a competitor to the sites on the plaque where Ca^{2+} binds to produce calculus. A chelate forming compound on the other hand will bind to Ca^{2+} in the saliva thus preventing the normal remineralisation of the teeth.

Although chlorhexidine is systemically well tolerated a concentration of from about 0.2% by weight can cause erosion of the mucous membrane. In order to prevent inconvenient side effects it is of advantage to aim at as low a concentration as possible of the active compounds in the dental preparation.

Clinical Test

In order to illustrate the effect of a dental preparation according to the invention a solution was prepared containing 0.05% by weight chlorhexidine and 0.04% by weight cupric acetate. A group of 15 healthy subjects took part in a double blind cross over study, wherein each subject took part in 3 studies which each lasted for three weeks. During each three week period the subjects performed no oral hygiene. As a positive control the subjects rinsed with a 0.12% by weight chlorhexidine solution for three weeks and as a negative control they rinsed with physiological saline solution. Plaque and gingivale indices were registered according to clinical standard methods (Loe, H. J. of Periodontology 36:610-616, 1965).

The results of the test have been compiled in the following table

TABLE

Subject	Plaque index			Gingivale index		
	Chlor-hexidine*	Chlor-hexidine** and cupric acetate	Physiological saline	Chlor-hexidine*	Chlor-hexidine** and cupric acetate	Physiological saline
1	0.68	0.95	1.00	0.56	0.76	0.95
2	1.12	0.98	1.83	0.89	0.89	1.00
3	0.65	1.42	1.75	0.95	1.23	1.52
4	0.89	0.71	1.62	0.91	0.77	1.11
5	0.68	0.96	1.75	1.32	1.24	1.30
6	0.89	0.67	1.78	1.03	1.21	1.16
7	0.39	0.52	1.27	0.75	0.70	1.27
8	1.02	0.40	1.54	0.38	0.35	0.38
9	0.50	0.71	1.51	1.12	1.03	1.21
10	0.86	0.98	1.47	1.32	1.14	1.05
11	1.04	1.27	1.75	0.87	1.05	1.00
12	0.54	1.10	1.86	1.03	1.56	1.49
13	0.70	0.56	1.59	1.13	1.13	1.06
14	0.35	0.81		0.91	1.09	
15	0.57	1.08	1.61	0.96	1.19	1.38
Mean value $\bar{x}$	0.73	0.88	1.60	0.94	1.02	1.38

* solution of 0.12 % by weight chlorhexidine in 11.6% by weight alcohol, pH 5.5 ("PERIDEX" from Procter and Gamble)

** solution of 0.04 % by weight cupric acetate and 0.05 % by weight chlorhexidine in destilled water prepared from 20% chlorhexidine gluconate ("HIBITANE")

The results show that the solution of chlorhexidine and cupric acetate in plaque formation inhibition was comparable with the solution of chlorhexidine solely, having more than twice the concentration.

Said two solutions were on their turn significantly more effective than the saline solution. In addition it is well known that a 0.05% by weight chlorhexidine solution does not bring about a clinical effect (Skørland

et al, Scand. J. Dent. Res. 86: 103-107, 1978).

The clinical study also showed that the chlorhexidine/cupric acetate solution gave less discoloration compared to pure chlorhexidine and that this mild form of discoloration could easily be removed by conventional tooth brushing.

In order to illustrate the synergistic effect between cupric acetate and chlorhexidine microbiological tests in vitro have been performed with Streptococcus mutans and Actinomyces viscosus, two common oral bacteria which are believed to be essential for the formation of caries and periodontitis. Currently available clinical methods are not accurate enough to unambiguously demonstrate a synergistic effect of a combination of chlorhexidine and a copper salt on plaque inhibition, as there is a great variation in the plaque growth between individuals. In combination with the clinical test above showing the effects of chlorhexidine solely and of a combination of chlorhexidine and copper salt of the invention, we however believe that the results from the following bacterial test can be used to prove the synergistic effect.

Test on bacteria

MIC (minimal inhibitory concentration) tests were performed with chlorhexidine and cupric acetate singularly and with different combinations of said salts on Streptococcus mutans and Actinomyces viscosus respectively. Percent values refers in the following to percent by weight if nothing else is stated.

Isolates of Streptococcus mutans serotype C and Actinomyces viscosus were cultured in Trypticase Soy Broth (TSB) supplemented with yeast extract (YE) (0.5%) in a 5% CO_2-incubator for 24 hours. The cultures were harvested by centrifugation, washed and resuspended in TSB-YE of the double strength and were adjusted to the appropriate optical density upon reference to a predetermined standard curve. Serial dilutions of chlorhexidine gluconate and cupric acetate were made in 0.15 M NaCl. The assay mixtures consisted of a standardized inoculate of washed cells (final concentration $5x10^5$ cfu/ml) and of the same volume of the compound to be tested. To determine an optional synergistic effect different concentrations of each compound were combined and inoculated as above. All tubes were incubated for 24 hours in 5% CO_2 at 37°C.

The results are given in Fig. 1 and 2. The MIC-value of chlorhexidine was 0.76×10^{-6}% (weight/volume) and of cupric acetate 0.02% (weight/volume).

In the first series of tests different concentrations of cupric acetate

were mixed with chlorhexidine gluconate as is shown in Fig. 1. The highest used concentration of cupric acetate was 0.02%, the previously determined MIC-value. Dilutions were made to a final concentration of $6{,}25 \times 10^{-4}$%. The concentration of chlorhexidine was held constant at 2.44×10^{-6}%, that is 0.25 x the MIC-value. It should be observed that a significant inhibition of the growth (30-60%) was obtained with the combinations of the two compounds at concentrations that allowed growth in tests with only one substance.

In the second series of tests a still more pronounced effect could be observed when cupric acetate in a concentration of 0.125 x MIC-value was mixed with chlorhexidine gluconate of a concentration varying from the MIC-value to 0.063 x MIC-value, as is shown in Fig. 2. At the lowest concentration tested, 0.125 x MIC-value of cupric acetate and 0.063 x MIC-value of chlorhexidine gluconate, a growth inhibition of 30-40% was achieved. Similar results were attained with A. viscosus isolate.

From this can be concluded that a combination of chlorhexidine and a cupric salt has a synergistic growth inhibiting effect on the tested bacterial strains.

A dental preparation according to the invention comprising an antimicrobal bisbiguanide in combination with a physiologically acceptable cupric salt in addition to an improved plaque inhibiting effect at a lower concentration also brings about a reduced discoloration of the teeth as well as a reduced calculus formation. A solution can contain 0.5 - 5.5 mM bisbiguanide in combination with 0.55 - 10 mM cupric salt, preferably 0.5 - 2.0 mM bisbiguanide and 2.0 - 5.0 mM cupric salt. A solid preparation preferably contains 2.1 - 21 mM bisbiguanide in combination with 0.55 - 100 mM cupric salt.

The dental preparation according to the invention can have the form of a mouth rinse, tooth paste or gel and also have the form of a tablet or a chewing gum. A mouth rinse can contain 0.05 - 0.5, preferably 0.05 - 0.2% by weight bisbiguanide as an acetate or gluconate and 0.01 - 1.0, preferably 0.04 - 0.08 % by weight cupric acetate or cupric gluconate. A tooth paste or gel should contain 0.2 - 2 percent by weight bisbiguanide as an acetate or gluconate and 0.01 - 10 percent by weight cupric acetate or cupric gluconate. A tablet or chewing gum should contain 0.2 - 2 percent by weight bisbiguanide as acetate or gluconate and 0.01 - 10 percent by weight cupric acetate or cupric gluconate.

CLAIMS

1. A dental preparation containing a water soluble, physiologically acceptable salt of an antimicrobal bisbiguanide, characterized in also containing a water soluble, physiologically acceptable cupric salt.

2. A dental preparation according to claim 1, characterized in comprising the salt of the bisbiguanide and the cupric salt in a proportion giving a synergistic plaque preventing effect.

3. A dental preparation according to claim 1 or 2, characterized in that the antimicrobal bisbiguanide is chlorhexidine.

4. A dental preparation according to any of claims 1-3, characterized in that the salt of the antimicrobal bisbiguanide is an acetate or gluconate salt.

5. A dental preparation according to any of claims 1-4, characterized in that the cupric salt is cupric acetate or cupric gluconate.

6. A dental preparation according to any of claims 1-5, characterized in that it is in the form of a mouth rinse, containing 0.05 - 0.5 percent by weight bisbiguanide as an acetate or gluconate and 0.01 - 1 percent by weight cupric acetate or cupric gluconate.

7. A mouth rinse according to claim 6, characterized in containing 0.5 - 2.0 mM bisbiguanide and 2.0 - 5.0 mM cupric salt.

8. A dental preparation according to any of claims 1-5, characterized in that it is in the form of a tooth paste or a gel containing 0.2 - 2 percent by weight bisbiguanide as an acetate or gluconate and 0.5 - 10 percent by weight cupric acetate or cupric gluconate.

9. A dental preparation according to any of claims 1-5, characterized in that it is in the form of a tablet or a chewing gum containing 0.2 - 2 percent by weight bisbiguanide as an acetate or gluconate and 0.01 - 10 percent by weight cupric acetate or cupric gluconate.

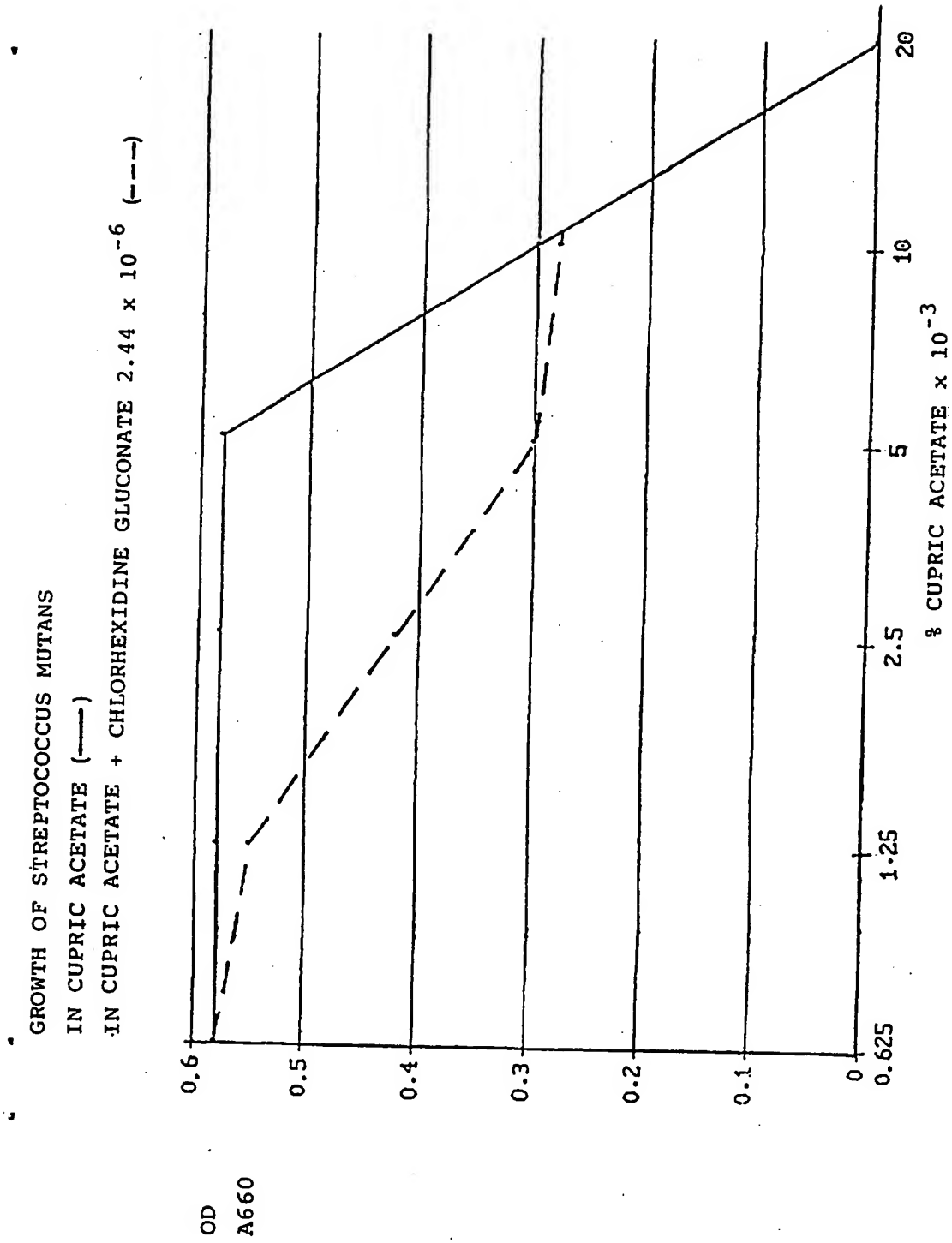

GROWTH OF STREPTOCOCCUS MUTANS
IN CUPRIC ACETATE (——)
IN CUPRIC ACETATE + CHLORHEXIDINE GLUCONATE 2.44 x 10-6 (– – –)
OD A660
0.6
0.5
0.4
0.3
0.2
0.1
0
0.625
1.25
2.5
5
10
20
% CUPRIC ACETATE x 10-3

FIG. 1

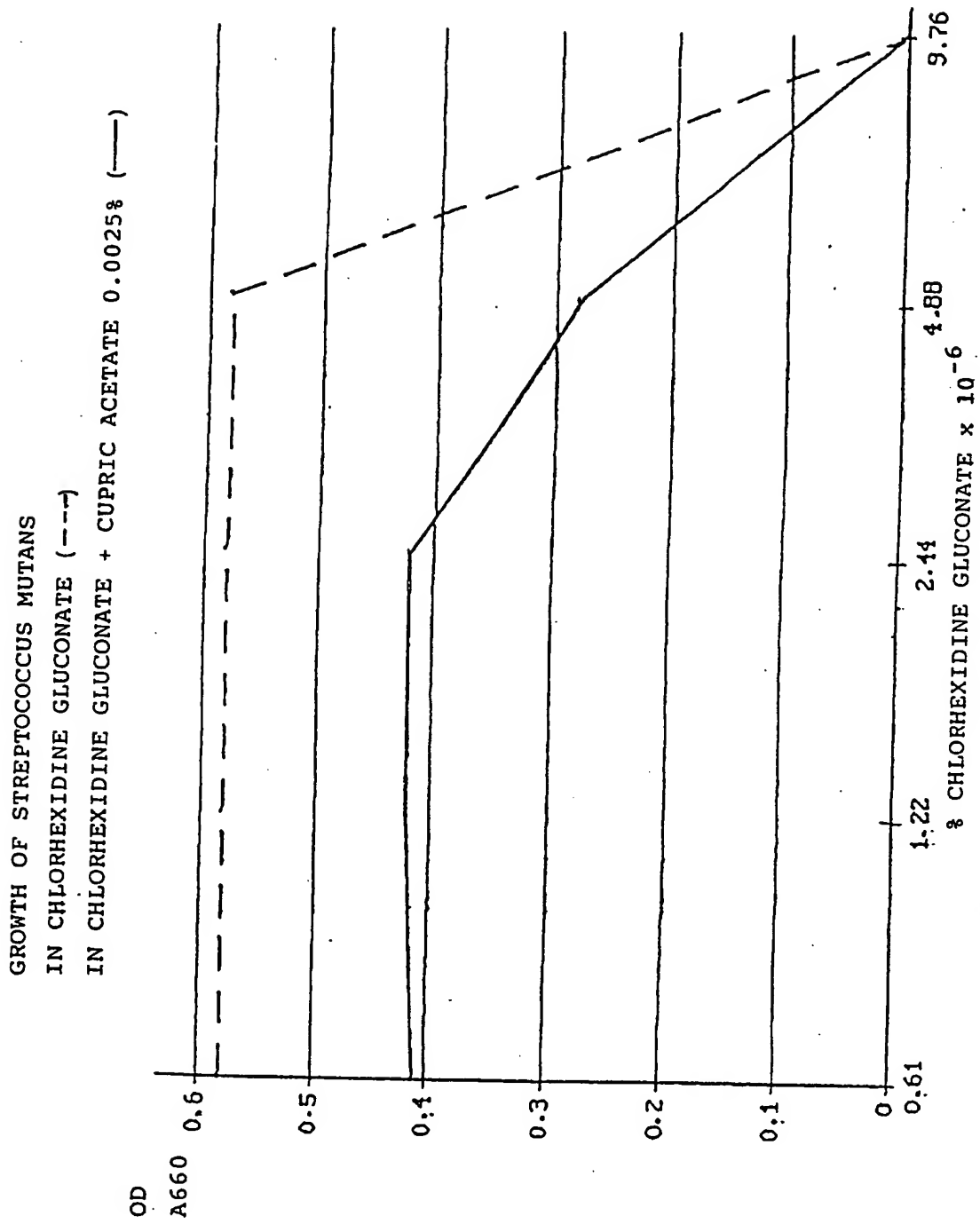

GROWTH OF STREPTOCOCCUS MUTANS
IN CHLORHEXIDINE GLUCONATE (---)
IN CHLORHEXIDINE GLUCONATE + CUPRIC ACETATE 0.0025% (——)
OD A660
0.6
0.5
0.4
0.3
0.2
0.1
0
0.61
1.22
2.44
4.88
9.76
% CHLORHEXIDINE GLUCONATE x 10^{-6}

FIG. 2

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE 90/00165

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: A 61 K 7/16

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
Y	EP, A1, 0229375 (BLENDAX-WERKE R. SCHNEIDER) 22 July 1987, see page 1, line 14 - line 17; page 1, line 39 - line 41 claims	1-9
Y	DE, A1, 2534887 (THE PROCTER & GAMBLE CO.) 26 February 1976, see page 1, line 9 - line 18; page 4, line 2 - line 31; page 7, line 15 - line 21	1-9
A	EP, A1, 0038867 (BLENDAX-WERKE R. SCHNEIDER GMBH & CO.) 4 November 1981, see the claims	1-9
A	EP, A2, 0216189 (BLENDAX-WERKE R. SCHNEIDER) 1 April 1987, see column 1, line 15 - line 21; column 2, line 21 - line 25 claims	1-9

* Special categories of cited documents:[10]

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12th June 1990	1990 -06- 18
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Dagmar Järvman

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.PCT/SE 90/00165

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on 90-05-07
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A1- 0229375	87-07-22	DE-A-	3600165	87-07-09
		JP-A-	62164614	87-07-21
		US-A-	4795628	89-01-03
DE-A1- 2534887	76-02-26	AT-B-	345988	78-10-10
		AU-D-	8342975	77-02-03
		BE-A-	832297	76-02-11
		CA-A-	1042806	78-11-21
		FR-A-B-	2281107	76-03-05
		GB-A-	1507846	78-04-19
		JP-A-	51041446	76-04-07
		NL-A-	7509469	76-02-11
		SE-A-	7508867	76-02-10
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		AU-D-	6941881	81-11-05
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		CA-A-	1149747	83-07-12
		JP-A-	56169619	81-12-26
		US-A-	4332791	82-06-01
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		JP-A-	62089614	87-04-24
		US-A-	4824661	89-04-25